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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/935,061	08/21/2001	Brian K. Kobilka	STAN-213	7757
24353	7590	04/19/2006	EXAMINER	
BOZICEVIC, FIELD & FRANCIS LLP 1900 UNIVERSITY AVENUE SUITE 200 EAST PALO ALTO, CA 94303			LI, RUIXIANG	
			ART UNIT	PAPER NUMBER
			1646	

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Please find below and/or attached an Office communication concerning this application or proceeding.

DETAILED ACTION

Status of Application, Amendments, and/or Claims

On further consideration, the finality of the rejection of the last Office action is withdrawn.

Applicants' amendment filed on 04/03/2006 has been entered in full. Claims 1-13 have been canceled. Claims 20-24 are pending and under consideration.

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office Action.

Withdrawn Objections and/or Rejections

The rejection of claims 1-4 and 9-12 under 35 U.S.C. 103(a) as being unpatentable over Dunham et al. (J. Biol. Chem. 274:1683-1690, 1999) in view of Gether et al. (The EMBO Journal 16:6737-6747, 1997) have been withdrawn in view of canceled claims.

The rejection of claims 1, 5-7, and 13 under 35 U.S.C. 103(a) as being unpatentable over Farrens et al. (*Science* 274:768-770, 1996) in view of Parola et al. (*Analytical Biochemistry* 254, 88-95 91997) have been withdrawn in view of canceled claims.

The objection to claim 8 has been withdrawn in view of canceled claim.

Claims Rejections under 35 U.S.C. 103(a)

(i). Claims 20-23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Dunham et al. (J. Biol. Chem. 274:1683-1690, 1999) in view of Gether et al. (The EMBO Journal 16:6737-6747, 1997).

Dunham et al. teach conformational changes in rhodopsin upon photoactivation using a series of rhodopsin mutants containing single reactive cysteine residues in the cytoplasmic side of helix F (3rd intracellular loop)(see Fig. 1; abstract; the middle of right column of page 1685), including the mutant V250C; such a conformational change exposes the cytoplasmic loops and allows transducin to bind and become activated (2nd paragraph of right column of page 1683). The cysteine mutants were studied in two ways, by measuring their reactivity to a cysteine-specific reagent (PyMPO-maleimide) and by labelling the cysteins with a fluorescence label (monobromobimane) followed by fluorescence spectroscopic analysis (Abstract). Since the fluorescence change was measured in a 4-mm black jacketed cuvette containing 0.08% D β M (a detergent; left column of page 1685), the rhodopsin receptor would be in a membrane of detergent micelles (see page 13 of the instant specification for definition) and attached to cuvette (a immobilization phase), via either the N-terminal portion or C-terminal portion. Dunham et al. also teach that the rhodopsin antagonist, 11-cis-retinal, is covalently bound in the middle of helices, inactivating the protein in the dark state. Light causes the isomerization of 11-cis retinal to the all-trans form and activates the receptor (page 1683). Dunham et al. further teach reactivity measurement of rhodopsin cysteine mutants with PyMPO-maleimide using sample wells (the 2nd and 3rd paragraphs of right

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column of page 1684, in particular, last line of the 3rd paragraph of page 1684; bottom of right column of page 1685; Fig. 3 and Fig. 4C), which are necessarily arranged in a specific order, i.e., an array.

Dunham et al. fail to explicitly teach a method of identifying a ligand of a G protein coupled receptor (GPCR), for example, for a hormone or neurotransmitter, by detecting a conformationally sensitive fluorescent probe located within the third intracellular domain of the GPCR.

Gether et al. teach a human $\beta 2$ adrenergic receptor, a member of the superfamily of hormone and neurotransmitter GPCR, has 13 Cys residues one of which is located within the third intracellular loop (Fig. 1).

Therefore, it would have been obvious to one having ordinary skill in the art at the time the invention was made to apply the method of Dunham et al. to a human $\beta 2$ adrenergic receptor to use PyMPO-maleimide, a cysteine-specific reagent, as a conformationally sensitive probe to detect the conformational change of a human $\beta 2$ adrenergic receptor and thus to identify a ligand of the human $\beta 2$ adrenergic receptor with a reasonable expectation of success. PyMPO necessarily labels cysteine 265, which is located within the third intracellular domain, without labeling the cysteines in the transmembrane domains due to the large size of the PyMPO molecule (Fig. 2 of Dunham et al.). One would have been motivated to do so because Dunham et al. teach that the

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conformational change described in the study is a conserved and primary step in the activation of GPCRs, such as β -adrenergic receptor (right column of page 1689), and that the approaches used in the study should be applied to measurement of conformational changes of other GPCRs (top of page 1690).

(ii). Claims 1 and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Farrens et al. (*Science* 274:768-770, 1996) in view of Parola et al. (*Analytical Biochemistry* 254, 88-95 91997).

Farrens et al. teach detecting photoactivated conformational changes in rhodopsin using spin-labelled double cysteine mutants. Each contains one cysteine at the cytoplasmic end of helix C (Cys¹³⁹) and one cysteine at various positions in the cytoplasmic end of helix F (see Abstract; Fig. 1). Farrens et al. also teach that rhodopsin has a site of V-8 proteolysis that is located within the conformationally sensitive third intracellular domain (see, e.g., Fig. 1). After V-8 digestion, rhodopsin was cleaved primarily into two large fragments: an N-terminal fragment (~27 kD) and a C-terminal fragment (~13 kD) on SDS-PAGE (Figs. 1 and 3). The various rhodopsin mutants for V-8 proteolysis (see, e.g., Fig. 3) are necessarily arranged in a specific order, i.e., an array.

Farrens et al. fails to teach a method of identifying a ligand of a G protein coupled receptor (GPCR), for example, for a hormone or neurotransmitter by detecting a

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conformationally sensitive protease cleavage product resulted from a cleavage site within the third intracellular domain of the GPCR.

Parola et al. teach a human $\beta 2$ adrenergic receptor, a member of the superfamily of hormone and neurotransmitter GPCR, has a site within the third intracellular loop, which can be cut by protease factor Xa (Fig. 1). After protease factor Xa digestion, the human $\beta 2$ adrenergic receptor was cleaved into two large fragments: an N-terminal fragment and a C-terminal fragment on SDS-PAGE (Fig. 5).

Therefore, it would have been obvious to one having ordinary skill in the art at the time the invention was made to apply the method of Farrens et al. to a human $\beta 2$ adrenergic receptor to use protease factor Xa proteolysis, as a conformationally sensitive probe to detect conformational change of a human $\beta 2$ adrenergic receptor and thus to identify a ligand of the human $\beta 2$ adrenergic receptor with a reasonable expectation of success. One would have been motivated to do so because human $\beta 2$ adrenergic receptor shares both homology in primary amino acid sequence and similarity in topology with rhodopsin as taught by Parola et al. (top of right column of page 88) and the conformational changes upon activation are conserved in all GPCRs that mediates the actions of extracellular signals of light, odorants, hormones, and neurotransmitters (see, top of page 768 and the end of the article of Farrens et al).

Conclusion

No claims are allowable.

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Advisory Information

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ruixiang Li whose telephone number is (571) 272-0875. The examiner can normally be reached on Monday through Friday from 8:30 am to 5:00 pm. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Brenda Brumback, can be reached on (571) 272-0961.

Communications via Internet e-mail regarding this application, other than those under 35 U.S.C. 132 or which otherwise require a signature, may be used by the applicant and should be addressed to [Brenda.Brumback@uspto.gov]. All Internet e-mail communications will be made of record in the application file. PTO employees do not engage in Internet communications where there exists a possibility that sensitive information could be identified or exchanged unless the record includes a properly signed express waiver of the confidentiality requirements of 35 U.S.C. 122. This is more clearly set forth in the Interim Internet Usage Policy published in the Official Gazette of the Patent and Trademark on February 25, 1997 at 1195 OG 89.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (571) 272-1600.



RUIXIANG LI, PH.D.
PRIMARY EXAMINER

Ruixiang Li, Ph.D.
Primary Examiner
April 15, 2006